Chapter 5

Discussion
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Chlorophyta borivilianum and Phyllanthus amarus both have medicinal value to cure numerous human ailments. Samples of these plants were collected from different geographical regions of India. These had given different accession numbers and maintained at Center for Transgenic Plant Development, Jamia Hamdard, New Delhi. The evaluation of genetic diversity of these plant samples using molecular and phytochemical markers was therefore, undertaken in this study to measure and document in these plants growing in different geographical regions.

Morphological markers

Characterization of the genetic variations is an essential first step towards executing any organized plant conservation or improvement programs. Morphological marker(s) have been routinely used to identify genetic diversity, but major disadvantages associated with these markers are; the limited number of morphological characters available for analysis and these characters are influenced by environmental factors. As a consequence, different plant genotypes cannot be distinguished (Staub and Meglic, 1993). Most of the morphological markers in P. amarus viz. plant height, leaflets per compound leaf, compound leaf per plant, length of compound leaf and seeds per compound leaf varied in the plant accessions collected from one location to another location. C. borivilianum

However, is a vegetative propagated medicinal plant and morphological variation found regarding length of leaf, number of leaf per plant, root breadth, root length, number of roots per plant and length of inflorescence. The coefficient of variation observed in C. borivilianum and P. amarus was 2.72-14.72 and 4.95-13.38, respectively. Similarly, large variability in leaf size, leaf arrangement, leaf area index, plant growth duration, fleshy root number and fleshy root size in C. borivilianum was recorded by various workers in their germplasm collections (Jat, 1993; Bordia et al., 1995; Jat and Sharma, 1996; Kothari and Singh, 2001; Bhagat and Jadeja, 2003). A wide range of variability in growth and yield-attributing characters was also recorded in 10 accessions of C. borivilianum collected from Maharashtra and Madhya Pradesh states (Kothari and Singh, 2001). Most of the morphological data like length of petiole in sago palm was highly variable (Kjaer et al., 2004). This variability is possibly more strongly controlled by edaphic or climatic
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Factors than by genetic factors. The studies on genetic diversity of sago palm indicate a high degree of independence between geographical data, morphological data and starch contents (Kjaer et al., 2004).

Correlation between DNA fingerprints and phytochemical fingerprints
Efforts to correlate phytochemistry with DNA data are at a preliminary stage for medicinal plants. The polymorphism based on phytochemicals in *P. amarus* at wavelength 254nm and 615nm was found 73.40% and 75.06%, while polymorphism based on DNA fingerprint was 62.52%. In *C. borivilianum*, the polymorphism observed based on phytochemicals at wavelength 254nm and 430nm was 76.19% and 75%, while polymorphism based on DNA fingerprint was 35.35%. This supports the contention that chemical characters can be used effectively to identify the genetic relationship among various plant species. Genes for secondary metabolism may in turn be derived from genes for primary metabolism by gene duplication and divergence or possibly also by allelic divergence (Pichersky and Gang, 2000). Domain swapping represents another mechanism for the creation of new composite genes (Pichersky and Gang, 2000; Doolittle, 1995). Approximately one-quarter of the genes in the *A. thaliana* genome (~5,000 genes) are predicted to be involved in secondary metabolism, and many of these are likely to have been recruited directly or indirectly from primary metabolism (*The Arabidopsis* Genome Initiative, 2000).

Causes of genetic diversity in *P. amarus* and *C. borivilianum*

*Phyllanthus amarus* is a widespread weed and found everywhere in India. The polymorphism in *P. amarus* as found in our experiment with AFLP fingerprint was 62.52% and 35.35% in *C. borivilianum*. In a widespread species, the conditions for divergence and local speciation are likely to exist at the geographical and ecological edges of the species distribution (Levin, 2003). Although geographic variation within widespread species is well reported in the literature (Holman et al., 2003), there are cases of widespread species exhibiting neither genetic nor phenotypic differences, and thus geographic distributions not always a good predictor of genetic divergence (Gitzendanner and Soltis, 2000). Processes of geographical divergence occur by isolating mechanisms, in part due to the restriction of gene flow between populations. Among subpopulations of
a widespread species, different ecological environments and independent evolution of populations through genetic drift may lead to divergence. The varied environmental conditions at collection sites in both genus i.e. *Chlorophyta* and *Phyllanthus*. Some regions are tropical and some are temperate. The environmental conditions at tropical regions were differing from temperate regions. The climatic factors are major contributing factors in shaping the vegetation in the prairie ecosystem in North America (Weaver and Fitzpatrick, 1934). A number of environmental factors may exert selection pressure, thus affecting genetic and phytochemical diversity; among these are freezing events, salinity, heat and drought.

**Phytochemical diversity**

In *P. amarus*, the number of compounds were varying in various organs under different environmental conditions. Each organ has numerous unique compounds with specific Rf values. Indeed, the biochemical response of an organism to a conditional perturbation can be characterized by its effect on the differential accumulation of individual metabolites (Raamsdonk et al., 2001). The number of compounds in root, stem, leaf and seed of *P. amarus* at 254 nm and 615 nm wavelengths were 74, 87, 158, 84 and 80, 128, 165, 84, respectively. The number of compounds in *C. horvilliamum* at 254 nm and 430 nm were 140 and 149, respectively. The enormous biochemical diversity displayed in the plant kingdom is estimated to exceed 200,000 different metabolites (Pichersky and Gang. 2000). It is also possible however, that enzyme isoforms arise with altered substrate specificity. Such changes in specificity could explain the vast number of different metabolites that occur in the plant kingdom. The need for multiple technologies reflects the technical difficulty of measuring metabolites owing to large variations in their relative concentrations and Chemical complexities. Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to genetic or environmental changes. The Linkage of functional metabolomic information to mRNA and protein expression data makes it possible to visualize the functional genomic repertoire of an organism. Even though the *A. thaliana* genome has been completely sequenced, over 30% of its genes are not functionally classified according to sequence similarities to other organisms, and only 9% have been experimentally characterized (Arabidopsis Genome Initiative, 2000). The phenotypic
characters in *P. amarus* and *C. borivilianum* are thus, only in approximation to meet the criteria of taxonomic study. There was much diversity in secondary metabolites within *P. amarus* organs (root, stem, leaf and seed). At wavelengths 254nm and 615nm the phytochemical diversity among organs of *P. amarus* on the basis of compound detection was found 34% and 31.25%, respectively. Unfortunately, metabolites have a much greater variability in the order of atoms and subgroups compared to the linear 4-letter codes for genes or the linear 20-letter codes for proteins. Therefore, they cannot be sequenced like genes or proteins using read-outs from one end to the other. The numerous unique compounds were found in root, stem, leaf and seed and each compound had its specific Rf value. The plant metabolites are characterized by an enormous chemical diversity, every plant having its own complex set of metabolites. Upon elicitation huge numbers of genes are activated, many of them involved in metabolite biosynthesis (Goossens and coworkers, 2003). When tobacco BY-2 cells were treated with methyl jasmonate and cDNA-AFLP (amplified fragment length polymorphism) transcript profiling was performed and it was found that 600 genes were differentially regulated by this elicitor. By linking this data to metabolite analysis in a time-course experiment, it was possible to build an ample inventory of genes that were already known or novel genes.

**Specificity of Primer**

We have found high specificity with three base extensions in EcoRI/MseI primer combinations in *P. amarus* and in *C. borivilianum* to reduce mismatching. The primer extension was used in selective amplification step. This study was supported by vandaceous orchid hybrids (C-values unknown) where, clean and reproducible AFLP fingerprints were obtained using a 3-base extension on the EcoRI primer (Chen et al., 1999). The reason of high specificity of AFLP priming reaction conditions that overcomes the usual mismatch annealing associated with RAPD reactions (Neale and Harry, 1994). The result was elucidated with three primer combinations and each primer gave approximate similar phenetic dendrogram. Similarly the same result have been reported with *Withania somnifera* where inter and intraspecific genetic variation of 35 individuals of *Withania somnifera* and 5 individuals of *Withania coagulans* were revealed by AFLP technique (Negi et al., 2002). Thus, it was found that each primer combination...
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generated a similar phenetic dendrogram and a single primer combination could broadly distinguish not only *W. somnifera* from *W. conguleus* but Nagori samples from Kashmiri. Similar results have been reported in Soyabean and Barley (Becker et al., 1995) using AFLP technology. The use of primer extensions extends the target for detectable base changes beyond the original sites (Becker et al., 1995). Thus, AFLP typically display a high level of polymorphism as compared with other marker technologies.

Reproducibility of AFLP fingerprinting

We have done our experiment in triplicate and in each experiment gave reproducible results. The fingerprinting of both the plants was much faster than HPTLC phytochemical fingerprinting. For coca identification using the 5-primer AFLP approach that was selective and faster as compared with analysis based on flavanoid chemotaxonomy and 132 known and unknown coca leaf accession were evaluated (Johnson et al., 2003). The polymorphic loci in *P. amarus* and *C. borusii* were found to be 72 and 35 with three primer combinations of EcoRI/Msel. The large number of polymorphic loci detected by AFLP in a relatively short period of time, which is referred to as a high multiplex ratio. The repeatability of the AFLP banding patterns was very high, providing credibility to the conclusions derived from the analyses. As a new molecular technique with few guidelines on the infinite number of ways in which unexpected results can arise, reproducibility is the only easy way of assessing the quality of the data (Karp et al., 1996).

The AFLP analysis has several advantages over other molecular techniques for the analysis of genetic diversity and the determination of genetic relationships (Mace et al., 1999). We have obtained reproducible results with three-primer combinations of EcoRI/Msel primer, because of the pure DNA isolated from different samples obtained from different geographical regions of India by modified CTAB method (Salim et al., 2007). This was in contrast to wheat where different AFLP patterns were observed and dependent on the source of DNA (Donini et al., 1997). Vos et al. (1995) emphasized the requirement of high quality DNA preparations for generating reliable AFLP fingerprints. Similarly, DNA extracted from leaves and cones was of high purity, yielding reproducible AFLP banding patterns. In contrast, the DNA extracted from pellets, the
banding patterns generated from it were not as reproducible due to incomplete digestion of the genomic DNA as Pellets contain numerous secondary metabolites (Barth et al., 1994), which may inhibit enzymatic digestion (Townsend et al., 2000). Unlike, RAPD, the AFLP profiles do not alter with minor variations in the experimental conditions.

Population differentiation in *Chlorophytum borivilianum*

It is a vegetative propagated plant and polymorphism was found 35.35%. The germplasm collections made from different states in India show the occurrence of wide genetic variability in terms of plant type, maturity period, growth and yield characters, and size and shape of fleshy roots (Maiti and Geetha, 2005). Although the species is cross-pollinated in nature, self-pollination is also feasible artificially (Maiti and Geetha, 2005). Beside, morphological variations in this species, the variation also found in seed germination. Several workers have reported a range of variation in seed germination percentage and about 13% seed germination was reported from Gujarat (Bordia et al., 1995), 11–24% from Rajasthan (Jat, 1993) and 25–30% from Madhya Pradesh (Shrivastava et al., 2001). The present authors also observed a great difference in seed germination in their experiments: seeds from wild habitat of Maharashtra state (Akot) show up to 50% germination, but from cultivated populations from Gujarat and Madhya Pradesh, there was only 5–15% germination, suggesting the significance of growing environment on seed development chemistry.

All 21 accessions grouped into one cluster at 70% similarity level. Further, this cluster is subgrouped. The accessions of Bhopal, Hoshiyarpur and Sangrur grouped into separate subclusters. The accession of Bhopal has low similarity to the accession collected from Sangrur and high similarity to the accession of Jamia Hamdard and it was 81.8% and 95.3%, respectively. The accession of Hoshiyarpur has low similarity with accession collected from Meerut and high similarity with accession of Karimnagar and it was 83.3% and 95.7%, respectively. The origin of Hoshiyarpur accession can be assumed from accession of Karimnagar. The accession of Sangrur has low similarity to the accessions collected from Nagaur, Kota and Hyderabad and maximum similarity to the accessions collected from Lucknow, Aligarh, Indore and Jamia Hamdard, respectively. However, the accession of Hoshiyarpur being distant from, Nagaur, Kota, Hyderabad and
Karimnagar but showed 95.7% similarity. The origin of Hoshiyarpur sample can be assumed from Nagaur, Kota, Hyderabad and Karimnagar accessions. The genetic diversity in most clonal plant populations is found to be high and even similar to populations of nonclonal plants (Ellstrand and Roose, 1987; Hamric and Godt 1989, Widen et al., 1994). The use of AFLP technology in phylogenetic and ecological studies is becoming increasingly popular because of its unique ability to detect polymorphisms within the genome without requiring prior sequencing information. Thus, Results from AFLP data sets have been largely concordant with other molecular markers (Powell et al., 1996). AFLP methodology has been used to assess genetic diversity in many crop plants like Lactuca (Hill et al., 1996), Soybean (Maughan et al., 1996), Lens (Sharma et al., 1996), Sunflower (Hongtrakul et al., 1997), Tea (Paul et al., 1997) and Barley (Russell et al., 1997). Biodiversity in rice, hopes and grapevine was successfully analyzed by AFLP and results were reproducible (Zhu et al., 1998; Cervera et al., 1998).

On the basis of phytochemicals, the accession of Hoshiyarpur made separate subcluster. The accession of Hyderabad showed 22.5% similarity to the accession of Sangrur. This result showed that the accessions of these locations were highly diverged. The Accession of Hyderabad showed high similarity to the accession of Nagaur and Kota. Accession of Meerut showed high similarity to the accession of Lucknow (73.1%) and low similarity to Aligarh (53.6%).

**Genetic diversity and adaptation**

*Chlorophytum borivilianum* is an endangered medicinal plant and low level of genetic diversity was found in comparison to non-vegetative propagated plants. This low level of genetic diversity does not facilitate to *C. borivilianum* in adverse conditions. The polymorphism was estimated at wavelength 254nm and 430nm in *C. borivilianum* and it was 76.19% and 75%, as compared to DNA based polymorphism that was 35.35%, and the difference between DNA and secondary metabolites based polymorphism was more. Thus, environment has considerable influence on expression of characters such as fleshy root yield and carbohydrate and protein content (Jat, 1993; Bhagat and Jadeja, 2003). The Abiotic and biotic processes acting upon isolated populations are thought to be a key factor in species divergence (Grant, 1981; Loveless and Hamrick, 1984; Levin, 2003), but
populations with low levels of genetic diversity may not be able to adapt to a changing environment (Ellstrand and Elam, 1993).

Genetic composition of plant has been shown to reflect climatic parameters in a relatively short time frame. The polymorphism was found in C. horiviliamii from 32.60-40.0% with EcoRI/MseI primer combination. The average polymorphism with three primer combinations was 35.35%. Since, it is a vegetatively propagated medicinal plant and hence, it has low polymorphism in comparison to non-vegetatively propagated medicinal plant as Azadirachta indica (69.8%) (Singh et al., 1999). Allard and coworkers (Allard, 1988; Perez de la Vega et al., 1994) have shown that within a 60-years period genetically distinct populations grown under similar environments often develop similar multi-locus allelic associations and conversely, genetically identical materials grown under different environments develops different multi-locus associations. Allelic frequencies may change within a few generations in a predominantly selfing plant, whereas in an obligate out-crossing species, such as E. angustifolia, discernible multi-locus associations would theoretically occur much more slowly (Still et al., 2005).

**AFLP data analysis in Phyllanthus amarus**
The present study revealed that there is a large genetic diversity in P. amarus according to environmental conditions. On an average the polymorphism and polymorphic loci in P. amarus was 62.52% and 72 detected with AFLP fingerprints, indicating a higher marker index ratio. This analysis revealed that the polymorphism varied from as high as 67.5% to as low as 59.45% within the Phyllanthus accessions. A high level of population differentiation may be explained by several factors, such as the species breeding system, genetic drift, demographic fluctuations, or the genetic isolation of populations (Hogbin and Peakall, 1999). The accessions collected from Hyderabad and Pantnagar showed maximum similarity and it was found to be 95.8%. The accession collected from Jammu showed 80%, 78.9% and 83.9% similarity to the accessions of Kathua, Bahu fort and Udhampur, respectively. When populations are small and geographically and genetically isolated from one another, genetic drift influences the genetic structure and increases differentiation among populations (Barrett and Kolun, 1991; Ellstrand and Elam, 1993).
All accessions were grouped into one main cluster. The accessions collected from Jammu were grouped into a separate subcluster. This accession showed much diversity to the accessions collected from Jammu and Kashmir state and had similarity of 80%, 78.9% and 83.9% to the accessions collected from Kathua, Bathu fort and Udhampur, respectively. The accession collected from Aligarh showed 86.3 and 86.5% similarity to the accessions collected from Hyderabad and Pantnagar and grouped into a separate subcluster. The accession collected from Chhindwana had low genetic similarity to the accession of Sagar and high similarity to the accession collected from Betul (57.7%). The accession of Kota showed low similarity to the accession of Kathua and maximum similarity to the accessions collected from Assam, Betul, Sagar, Dehradun, Indore and it was 90.4%, 88.2%, 84.3%, 84.3% and 82.4%, respectively. The, RAPD which is non reproducible DNA fingerprinting and profiling of 33 collection of P. amarus from different locations covering states of Tamilnadu, Karnataka, Maharashtra, Gujarat, Assam, West Bengal, Tripura, Uttar pradesh, Panjab and Haryana and variations among these accessions with this marker was found 65% polymorphism. However, intra-population variation was found to be much larger in the accession from the southern part of country (Jain et al., 2003) and such a wide range in similarity coefficient values suggests that the Phyllanthus germplasm collection represents a genetically diverse population. One of the major contributory factors to the high degree of polymorphism observed in P. amarus may be on account of its evolutionary status as on out-crossing.

The polymorphism based on phytochemicals was higher than DNA based fingerprint. At wavelength 254nm, polymorphism in P. amarus was found 55.56% (root), 68.75% (stem), 86.95% (leaf) and 82.35%, respectively. Similarly at wavelength 615nm, polymorphism was 65% (root), 78.57% (stem), 83.34% (leaf) and 73.33% (seed), respectively. The average polymorphism at wavelengths 254nm and 615nm in P. amarus was 73.40% and 75.06% in comparison to DNA based fingerprint that was found to be 62.52%. It may be assumed that inclusion of individuals from other areas would result in higher values of genetic variation. Comparative DNA sequence/chromotaxonomic phylogenetic trees showed that the chemical characters of the investigated species were able to generate essentially the same phylogenetic relationships as the DNA sequences.
under the same environmental conditions (Jiang et al., 2006). The DNA based markers are highly reliable and it was explained in hop plant in which DNA was extracted from different developmental stages and from different organs of a single hop plant showed identical AFLP banding patterns.

Using DNA sequence data, we were able to produce a phylogeny of the medicinal plant *C. horivilianum* and *P. amarus*. This DNA based phylogeny matched more closely to the phylogeny produced from chemical markers obtained from metabolic profiling experiments using extracts from root (*C. horivilianum*) and four organs of *P. amarus* (root, stem, leaf, seed). These chemotaxonomic investigations cannot be very suitable for specific groups of plants and can uncover relationship that matches those determined by DNA molecular data. Further, identification and quantification of the actual bioactive compounds in the samples, that depends upon gene/ enzyme expression under different environmental conditions (Jiang et al., 2006).

Likewise, the general consensus among the dendrograms produced by the different similarity coefficients and clustering methods showed that the data is robust and strengthens confidence in the results. The AFLP analysis revealed quite high level of variation in the plant produced by seeds (*P. amarus*). Similarly, a high level of variation was found in the taxa of section *Stramonium*, even though there is very little variation within taxa (Beath, 1987).

**Content of Sarsapogenin and Phyllanthin**

Potential variation of chemical variation in accessions of *C. horivilianum* and *P. amarus* from different geographical regions was more. Two bioactive constituents Sarsapogenin and Phyllanthin were quantitatively determined. Calibration curves were derived from independent spot of concentration of Sarsapogenin and Phyllanthin versus the peak area. Linearity was found in concentration range between 500 to 2000 ng/ml for Sarsapogenin and 100 to 500ng/ml for phyllanthin with high accuracy and reproducibility. Regression analysis of the experimental data points showed a linear relationship with excellent correlation coefficient ($r^2$), which was 0.991 and 0.995 for Sarsapogenin and Phyllanthin, thus suggesting high precision in this analysis. The linear regression equations for the curve for sarsapogenin and phyllanthin concentration were $Y = 465.782 + 5.293 \times X$ and
\[ Y = 788.980 + 24.302 \times X \] respectively where \( x \) was the concentration of standard sarsapogenin and phyllanthin (ng/ml\(^{-1}\)) and \( y \) was the total peak area. Precise information on the estimation of sarsapogenin has not yet been established due to non-availability of genuine standards. Steroidal sapogenins are reported to be the active principle present in other species of the genus *Chlorophytum* (Xing-Cong et al., 1990; Tandon et al., 1992; Tandon and Shukla, 1992, 1995, 1996, 1997; Qiti et al., 2000). The mean content of sarsapogenin (% DW) in root of *C. borivilianum* was (0.1872-0.9888%) and phyllanthin in *P. amarus* was (% DW) 0.0026-0.0194% (root), 0.0088-0.2056% (stem), 0.0613-0.387(leaf) and 0.0294-0.2467% (seed) summarized in Table 1. Thus, the simultaneous identification and quantification of metabolites is necessary to study the dynamics of the metabolome, to analyze fluxes in metabolic pathways and to decipher the role of each metabolite following various stimuli. The challenge of metabolomics is to find changes in the metabolic network that are functionally correlated with the physiological and developmental phenotype of a cell, tissue or organism (Sweetlove et al., 2003). We determined the content of these compounds with HPTLC for 21 accessions of *C. borivilianum* and 19 accessions of *P. amarus*. The roots of *C. borivilianum* are reported to contain 42% of carbohydrates, 8-9% of proteins, 3-4% fibres, 2-17% saponin and 2% sapogenin (Bordia et al., 1995). There was large variability in the content of these compounds in different accessions collected from different geographical regions of India. The phyllanthin content was higher in tropical regions in comparison to temperate regions. Tropical regions including Agra, Hyderabad, Rajsthan, Indore, Sagar, Betul, Guna, Chhindwana and Jamia Hamdard, respectively. Similarly the sarsapogenin content was also higher in samples of tropical regions viz., Indore, Guna, Bhopal, Nagpur, Akola, Jalgaon, Sangrur, Hoshiyarpur, Nagaur, Kota, Hyderabad and Karimnagar except Betul in comparison to temperate regions. Variation in expression or activity of genes/enzymes involved in the production of these metabolites can explain these differences (Jiang et al., 2006). The phyllanthin content was varied in different organs of *P. amarus*. It was higher in leaf in comparison to other organs. The minimum phyllanthin content was found in root organ. Similarly, Artemisinin has been detected from aerial parts of the plant, mostly in leaves and inflorescences with low levels in stems and none in pollen or roots (Ferreira and Janíck, 1996). However, the sarsasaponin biosynthesis is a multistep process that is
not well understood for any plant species, and the genes for complete pathways have not
been cloned (Haralampidis et al., 2001). Among all the species of Chlorophytum present
in India, C. borivilianum produces the high yield of roots along with the highest saponin
content (Bordia et al., 1995). However, the saponin content was found to be affected by
genotype and environment. When same accessions were collected from forest and
cultivated at sandy loam soil at CTAE, Udaipur and clay loam soil of RCA, Udaipur
showed varied response of genotypes to the locations, in terms of saponin content.
Genotype RC-14 yielded saponin as high as 9.3%, while at the same site other genotype
RC-28 yielded only 1.8% saponin (Kaushik et al., 2005). It was also found that the
environment has considerable influence on expression of characters such as fleshy root
yield and carbohydrate and protein content (Jat, 1993; Bhagat and Jadeja, 2003).

In our results the phyllanthin as well as sarsapogenin almost were higher in those
accessions, which were collected from tropical regions of India. In contrast to these
phytoconstituent, emodin has a worldwide distribution, occurring in subtropical and
tropical families (Bignoniaceae and Simaroubaceae), that mainly inhabit the temperate
regions (Polygonaceae and Saxifragaceae), and in families inhabiting both the tropics and
the temperate regions (Rhamnaceae and Clusiaceae). Emodin was originally reported as
being more common in barks and roots (Evans, 1996), but it is clear now that emodin is
present in other vegetative organs (stem, foliage) as well as in reproductive organs
(flower, fruit, seeds, pods). The concentration of emodin in different plant organs is
frequently unequal, a phenomenon found in many secondary metabolites (Bernays and
Chapman, 1994). The flowers and roots of Rumex luminiastrum contain higher emodin
concentration than the leaves (Abd El-Fattah et al., 1994). Synthesis and accumulation of
secondary metabolites in plants is regulated in space and time and is affected by abiotic
environmental factors, such as light intensity, soil minerals, osmotic stresses (drought and
salinity), seasonality (Waterman and Mole, 1994). Abiotic environmental factors that
constrain the production of secondary metabolite in plants indirectly influence the
interactions of plants with their biotic environment (Waterman and Mole, 1994). The
strongest inhibition of viral DNAp of extract of P. amarus was obtained from warm
environment; thus a tropical side was desired (Unander et al., 1990). The DNAp
inhibitory activity was relatively unaffected by soil condition, but differed among accessions, suggesting that genetic variability does exist for this trait (Unander et al., 1990). The research work is in progress to elucidate the structure and biological activity of secondary metabolites present in these medicinal plants.

Authentication of local market samples of *Chlorophytum borivilianum*

AFLP fingerprint

The developed phytochemical and AFLP fingerprint for *Chlorophytum borivilianum* collected from wild conditions was assumed as a standard for authentication. The authentication of market samples was done with same primer combination as applied for samples collected from wild conditions. The samples purchased from Delhi and Aligarh showed similar AFLP fingerprint as fingerprint generated from accessions collected from Aligarh and Chandanpur sanctuary. The samples purchased from Meerut showed similar fingerprints as fingerprints generated from samples collected from Nagpur and Akola. The total number of bands were found to be 78 with two primer combinations (EcoRI/MseI primer-2 and primer-3) for market samples. The monomorphic, polymorphic and unique loci were 65, 9 and 4 that was identical with accessions collected from Aligarh, Chandanpur, Nagpur and Akola with same primer combinations.

Phytochemical fingerprint

Phytochemical fingerprints showed few similarities in compounds to the accessions collected from wild conditions. At 254nm wavelength, the sample purchased from Delhi showed phytochemical similarity to the accessions of Hoshiyarpur and Nagaur. The accession of Hoshiyarpur has two additional compounds of Rf values 0.92 and 0.88 that was absent in sample of Delhi (C1). Similarly, the Nagaur accession has one additional compound that was absent in sample of Delhi (C2). The sample purchased from Meerut showed similarity to the accession of Dehradun but it has one additional compound of Rf value 0.93. The sample purchased from Aligarh showed similarity to the accession of Kota but it has one additional compound of Rf value 0.41. At wavelength 430nm, the samples purchased from Delhi, Meerut and Aligarh almost showed similarity in phytochemicals except samples of Aligarh. These samples have an additional compound of Rf value 0.63. There was no adulteration found in samples purchased from market.
Phyllanthus amarus

AFLP fingerprint

The sample purchased from Delhi showed similarity to the sample collected from Gorakhpur and Agra. The samples purchased from Aligarh showed similarity to the accessions collected from Gorakhpur and Kota. Similarly, the samples purchased from Agra showed similarity to the accession collected from Kota. However, the AFLP fingerprint of samples purchased from Delhi, Aligarh and Agra had only similarity with AFLP fingerprint of accessions collected from Kathua and Bahu fort, Agra and Aligarh, Kota and Aligarh respectively. The total number of bands was found to be 51 with two primer combinations (EcoRI/Msel primer–1, Primer-3) for samples purchased from Delhi, Aligarh and Agra. The monomorphic, polymorphic and unique loci were 14, 27 and 3 that were identical in accessions collected from Gorakhpur, Agra and Kota respectively. Similarly, the genetic variability of P. grandis Cramer, P. barbatus Andr. and P. ornatus Codd was analyzed with two sets of AFLP primers allowing detection of 241 loci. A total of 22 monomorphic loci were identified in P. barbatus, 15 in P. grandis and 30 in P. ornatus. Among these, 5 loci were informative and species-specific to P. barbatus, 3 to P. grandis and 2 loci were unique to P. ornatus (Passinho-Soares et al., 2006). The authentication of Panex ginseng and Panex quinquefolius was done using amplified fragment length polymorphism (AFLP) and direct amplification of minisatellite region DNA (DAMD) by developing unique marker (Ha et al., 2002).

Phytochemical fingerprint

Phytochemical fingerprints showed few similarities in compounds to the accessions collected from wild conditions. At 254 nm wavelength, the samples of seed (P4, P5, P6), stem (P1, P2, P3, P4, P5, P6) and root (P1, P3, P4, P5, P6) have additional compound of Rf value 0.01. The samples purchased from Delhi and Aligarh has an additional compound of Rf value 0.93 in leaf (P1, P3), and in seed (P1, P2, P3). Similarly, the additional compound of Rf value 0.91 was present in Root (P4, P5, P6) purchased from Aligarh and Agra respectively.
At wavelength 615nm, the samples of Delhi and Aligarh have additional compounds of RF value 0.03 in root (P1, P2, P3) and the compound of RF value 0.03 was present in all samples of market that was absent in wild plants. The compound of RF value 0.98 was absent in all samples of stem that was present in stem of wild plants. Similarly, the compound of RF value 0.95 was absent in root samples (P1, P3) that was present in wild accessions respectively. The additional compound of RF value 0.1 was present in root samples of local market except samples of Agra (P6) that was absent in samples of wild conditions.

A great variability exist in phytochemical markers as they showed more diversity in comparison to AFLP markers. However, phytochemical markers are influenced by environmental conditions but DNA based markers are not influenced by environmental factors. A large similarity was observed between the samples obtained from local markets and those from wild sources. There was no adulterant found in C. borivilianum and P. amarus samples collected from local markets. However, there are some adulterants found in C. borivilianum and P. amarus in local markets. Curculio orchidodes (Kali musli) and C. arundinaceum adulterated in C. borivilianum, and P. debilis and P. fraternus in P. amarus (Webster, 1994). The P. amarus is usually misidentified with the closely related P. niruri L. in appearance, phytochemical structure and history of use. The P. niruri reaches a length of 60 cm, the fruits are larger, and the seeds are dark brown and warty (Morton, 1981). These adulterants had low medicinal efficacy and therefore degrade the medicinal efficacy of genuine samples.

Comparison of molecular markers
Morphological markers showed a wide variation in accessions collected from different geographical regions of India. The phenotypic coefficient of variation was found 2.72 (leaf length)-14.72(root width) in C. borivilianum while in P. amarus it was found 4.95(plant height)-13.38 (length of compound leaf). The highest Pearson correlation value \((r = 0.911)\) was recorded between combined and phytochemical markers which suggested that these markers contribute maximum in genetic diversity produced. The high Pearson correlation of combined molecular markers with phytochemicals \((r = 0.911)\) followed by AFLP \((r = 0.709)\) indicate that phytochemicals have relatively more
contribution in revealing genetic diversity. AFLP and phytochemicals data generated low Pearson coefficient value ($r=0.423$) which indicate that these two markers are giving polymorphism at different loci for the same accession.

Similarly, in *Phyllanthus amarus* highest Pearson correlation value ($r=0.797$) was recorded between combined and AFLP which suggested that these markers contribute maximum in genetic diversity produced. The high Pearson correlation of combined molecular marker with AFLP ($r=0.797$) followed by phytochemicals ($r=0.756$) indicate that AFLP marker have relatively more contribution in revealing genetic diversity. AFLP and phytochemicals data generated low pearson coefficient value ($0.243$) which indicate that these two markers are giving polymorphism at different loci for the same accession.

In all the cases, significant positive correlation was recorded with variable coefficient value. This is not surprising as these markers are known to target different genomic fractions involving repeat and/or unique sequences which may have been differentially evolved. In present study, according to the expectations, both markers generated somewhat similar pattern of genetic relationships. It may be concluded that both markers were somewhat similar to each other so there is a need to emphasize the use of the combination of these two marker systems for a comprehensive genetic analysis, while comparing the distance matrix generated from the combination of two markers (phytochemical and AFLP) with that of individual marker, although the correlation between molecular markers and morphological traits was non-significant. The result thus indicated that the pattern of relationships obtained from the combined marker assay is more reliable, followed by AFLP and phytochemicals individually.